

Deep Insight Section

The Hippo Kinase Pathway: a master regulator of proliferation, development and differentiation

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Abstract

Hippo signaling transduction pathway is widely conserved through evolution and controls cell growth, homeostasis, apoptosis, commitment, differentiation and senescence. It consists of a conserved kinase cascade whose final targets are the transcriptional coactivator Yorkie (Yki) in *Drosophila* and the homologues YAP and TAZ in mammals. These transcriptional coactivators are unable to bind DNA *per se*, and can regulate the activity of their target genes only in association with transcription factors. In *Drosophila*, Yki associates with the transcription factors Sd and Hth regulating pro-proliferative and anti-apoptotic genes. In mammals instead, YAP/TAZ can associate with several distinct transcription factors. This depends from the type of signals to which cells are subjected, the cell type and the developmental stage. The transcriptional outcome resulting from this association can be either pro-apoptotic or pro-proliferative. Hippo pathway dysregulation has been associated with several pathologic conditions (tissue overgrowth, developmental defects and cancer). In particular, solid tumors show an upregulation or hyperactivation of YAP/TAZ, while several hematologic tumors are associated with YAP downregulation. This might suggest that the Hippo pathway holds the potential to be an attractive target for novel therapeutic approaches for cancer.

Introduction

Hippo signal transduction pathway is an evolutionary conserved pathway, from flies to humans, that controls organ size, development, tissue regeneration-homeostasis and stem cell self-renewal through the regulation of cell proliferation, cell commitment and apoptosis. Components of the pathway include membrane associated proteins that sense cell polarity, cell density and mechanical cues, that in turn activate a cascade of kinases whose final target is the transcriptional coactivator Yorkie (Yki) in *Drosophila*, or its mammalian counterparts Yes Associated Protein (YAP) and Transcriptional coactivator with PDZ-binding motif (TAZ, also called WWTR1). These factors are unable to bind DNA *per se*, but can regulate

transcription in association with other transcription factors.

Aberrant regulation of the Hippo pathway is associated with tissue overgrowth and various types of cancers in mammals (see below). Thus, a major comprehension of the mechanisms at the basis of YAP/TAZ upstream regulation and downstream transcriptional response could also be relevant for the characterization of prognostic factors in cancer and for the development of novel anti-cancer therapies.

Hippo pathway core components

The core components of the Hippo pathway were firstly discovered in *Drosophila melanogaster* by mosaic genetic screens which showed a strong overgrowth phenotype shared by loss-of-function

mutants. Based on these findings, the Hippo pathway had been defined as an oncosuppressor pathway. In parallel, homologous components of the pathway were discovered in other organisms, including mammals (reviewed in Varelas and Wrana, 2012). Some of them are able to rescue mutant phenotypes in flies (Lai et al., 2005; Tao et al., 1999; Wu et al., 2003). The Hippo pathway core components are listed in table 1 and schematically represented in Figure 1.

They include two serine/threonine kinases associated with adaptor proteins: the first is the STE20 kinase Hippo (Hpo) with the adaptor protein Salvador (Sav) (MST1/2 and Sav1 in mammals) (Callus et al., 2006; Harvey et al., 2003; Jia et al., 2003; Kango-Singh et al., 2002; Pantalacci et al., 2003; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003), and the second is the NDR kinase Warts (Wts) associated with the scaffold protein Mats, (Lats1/2 associated with Mob1 in mammals) (Callus et al., 2006; Chan et al., 2005; Praskova et al., 2008; Wu et al., 2003). *Drosophila* Hpo and mammalian MST1/2 directly bind Sav protein and are able to phosphorylate and activate Sav itself and Mats (Sav1 and Mob1 mammalian counterparts). *Drosophila* Mats and mammalian Mob1 interact with and phosphorylate Wts and Lats1/2, respectively. Wts-Mats and Lats1/2-Mob phosphorylate in turn specific residues of the transcriptional coactivator Yki and its mammalian counterparts YAP and TAZ. Yki and YAP/TAZ phosphorylation result in their cytoplasmic sequestration via 14-3-3 binding (Dong et al., 2007; Hao et al., 2008; Kanai et al., 2000; Lei et al., 2008; Vassilev et al., 2001; Zhao et al., 2007), which inhibits TAZ/YAP nuclear functions as transcriptional coactivators, while promoting their cytoplasmic role (Varelas et al., 2010) or their proteasomal degradation (Liu et al., 2010; Zhao et al., 2010).

It is becoming clear that not only Hippo pathway core kinases are able to regulate YAP and TAZ nuclear activity. For example, recently it has been shown that SIRT1 protein is able to activate YAP2 isoform by deacetylation in hepatocellular carcinoma cells (HCC) (Mao et al., 2014).

Moreover, YAP and TAZ are at the crossroad between several other signalling pathways as Wnt, Tgf β and Notch (reviewed in Barry and Camargo, 2013). Conversely, Hippo pathway core components may be involved in cell cycle control independently of YAP/TAZ regulation. For example, Mst1 has been shown to promote apoptosis in injured cardiomyocytes independently of YAP phosphorylation (Maejima et al., 2013). In this case, Mst1 has been shown to phosphorylate beclin1, a protein that alternatively binds Atg14L-Vps34 or Bcl-2 protein. In normal conditions, beclin1 complexes with Atg14L-Vps34 to promote autophagy, a process required for the recycling of macromolecular proteins and damaged organelles. Meanwhile, Bcl-2 sequesters Bax and inhibits apoptosis. Mst1 phosphorylates Beclin1 at Thr108 during cellular stress. This causes Beclin1 dissociation from Atg14L-Vps34 and its association with Bcl-2 that is no more able to sequester Bax. This in turn leads to apoptosis.

Upstream regulators of Hippo pathway core components

Proteins involved in cell junction, cell polarity and G-protein-coupled receptor (GPCR) signalling are upstream regulators of the core Hippo pathway. These proteins regulate YAP/TAZ nuclear activity in response to both mechanical and biochemical stimuli originated from the extracellular matrix (ECM).

Cell junction/cell polarity: in vivo, epithelial cells are in contact each another through specialized cellular junctions, forming sheets that line the surface of the animal body and internal cavities (for example digestive and circulatory cavities). These cells are oriented in the space with an apical-basal polarity: the apical membrane is oriented to the outside surface of the body, or the lumen of internal cavities, and the basolateral membrane is oriented away from the lumen. Polarity proteins associate with junction proteins in order to contribute to their proper localization and assembly and thereby to the functional organization of the tissues.

Mammalian	<i>Drosophila</i>	Junctional localization	Cytoskeleton interaction
MST1/2	Hpo (Hippo)		v
Sav1	Sav (Salvador)		
Lats1/2	Wts (Warts)		v
Mob1a, Mob1b	Mats		
YAP/TAZ	Yki	v	

Table 1: Hippo pathway core components

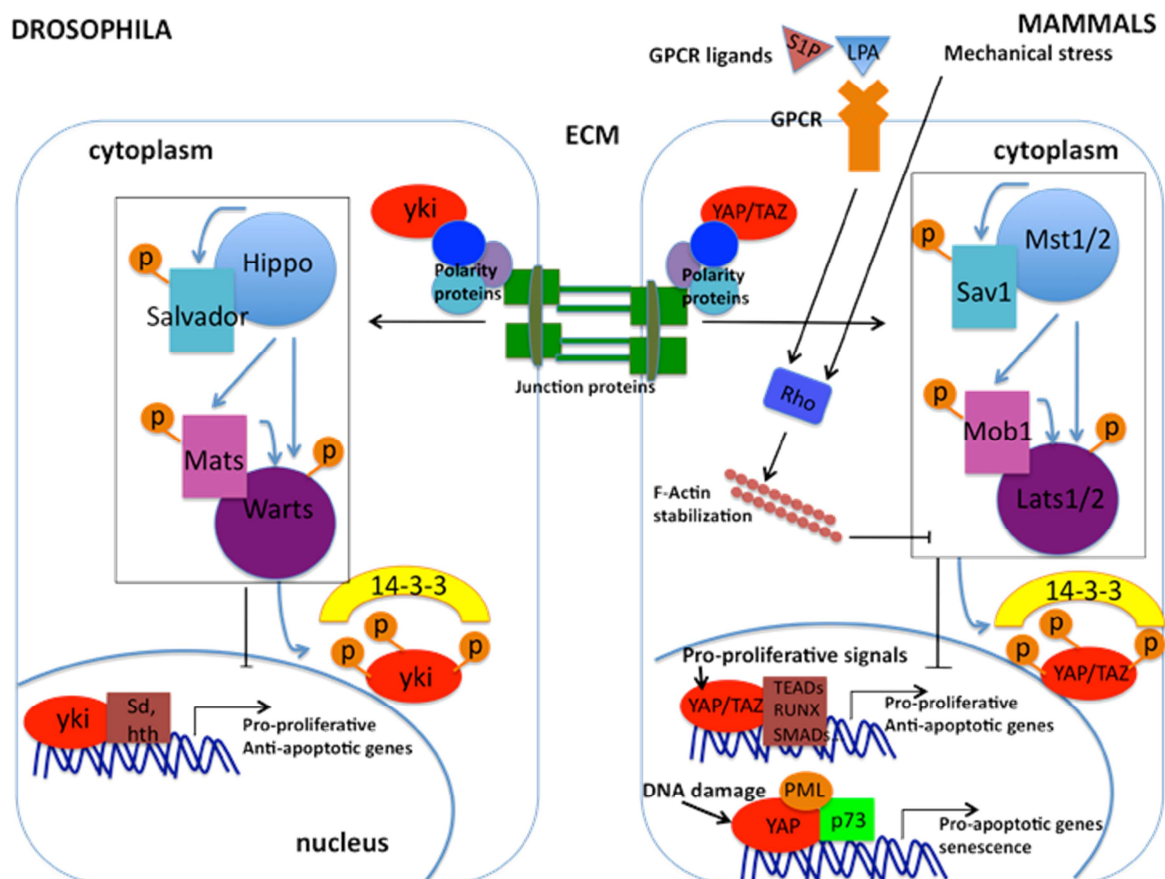


Figure 1. Schematic representation of Hippo pathway core components, their upstream regulators and transcriptional outcome in *Drosophila* (left) and mammals (right). In figure1, the Extracellular Matrix (ECM), the cytoplasm and the nucleus of cells are represented. Proteins are represented in various colours, with homologous components between *Drosophila* and mammals represented with the same colour. Black arrows indicate activation, while blunt lines indicate inhibition. Light blue arrows indicate phosphorylation of proteins by kinases. Orange balls indicate phosphorylation sites of target proteins. The Hippo pathway core kinase cassette is represented inside a black rectangle. For simplicity, junction proteins and polarity proteins are not represented by the specific *Drosophila* or mammalian subunits. In general, even if they are represented by different complexes in *Drosophila* or mammals, either homologous or not, they inhibit Yki and YAP/TAZ nuclear activity by sequestering them at the apical membrane or by interacting with and activating the Hippo pathway core kinases (represented in the black rectangle) that in turn inhibit Yki and YAP/TAZ nuclear activity. In mammals, GPCR signalling and mechanical stress coming from the ECM activate Rho GTPase that in turn stabilizes the actin cytoskeleton thus inhibiting Hippo pathway core kinases (and activating YAP/TAZ nuclear activity). In the nucleus, *Drosophila* Yki interacts with Sd or Hth transcription factors and activates pro-proliferative and anti-apoptotic genes. Mammalian YAP and TAZ instead interact with several different transcription factors (see also table 3) and the resulting transcriptional outcome may be either pro-proliferative or pro-apoptotic. This might depend from the incoming signals to which cells are exposed and from the specificity of the associated transcription factor.

The Kibra complex, conserved in *Drosophila* and in mammals, represents an example of apical proteins involved in Hippo pathway regulation. It recruits Hippo pathway core components like Hpo and Sav to the apical plasma membrane for activation, thus inhibiting YAP/TAZ nuclear activity and tissue growth (Genevet et al., 2010; Yu et al., 2010). Also the Crumbs polarity complex, the Scribble complex and Par3 polarity complex have been shown to be negative regulators of YAP and TAZ nuclear function (Chen et al., 2010; Gurvich et al., 2010; Ling et al., 2010; Robinson et al., 2010; Varelas et al., 2010). Other polarity proteins as Ajuba and LKB1 have been shown to negatively regulate YAP and TAZ nuclear function (Das Thakur et al., 2010; Nguyen et al., 2013). Moreover, many cell junction associated proteins, such as angiomotin (AMOT),

MPDZ, PATJ, PALS1, LIN7C, PTPN14, ZO-1, α - β -catenin and E-cadherin have been identified as interacting partners or regulators of Hippo pathway core components (Kim et al., 2011; Liu X et al., 2013; Oka et al., 2010; Remue et al., 2010; Schlegelmilch et al., 2011; Zhao et al., 2011).

In general, these proteins negatively regulate YAP/TAZ nuclear function by sequestering YAP/TAZ to the apical plasma membrane, thus excluding them from the nucleus, and by interacting with and activating hippo pathway core kinases. This in turn inhibits YAP and TAZ nuclear function by phosphorylation (Genevet et al., 2010; Varelas et al., 2010; Yu et al., 2010; Zhao et al., 2011). Indeed, disruption of cellular junctions or downregulation of cell polarity/cell junction proteins leads to YAP/TAZ activation (Chen et al.,

2010; Cordenonsi et al., 2011; Dupont et al., 2011; Varelas et al., 2010). Thus, YAP and TAZ nuclear function is inhibited by cell contact to finely tune the proliferation of cells within a tissue and an organ during physiological tissue-organ growth and regeneration. However, there are few exceptions to this rule: NPHP4 (nephronophthisis 4) can interact with and inhibit Lats1 (Habbig et al., 2011) and ZO-2 (zona occludens-2) can induce YAP nuclear localization (Oka et al., 2010). The regulation of Hippo pathway by apical-basal polarity and cell junction is largely conserved in *Drosophila* and mammals, even if not all the proteins are conserved between flies and vertebrates (Bossuyt et al., 2014). **Biochemical signals:** very recently, several groups have shown that diffusible signals and metabolites like LPA, S1P, thrombin and statins regulate YAP/TAZ function (Miller et al., 2012; Mo et al., 2012; Sorrentino et al., 2014; Yu et al., 2012). LPA, S1P and thrombin activate G-protein coupled receptor (GPCR) which usually activate downstream signalling through heterotrimeric G proteins that in turn activate the mediator Rho GTPase. Depending on which G α protein is activated, YAP and TAZ may be either activated or repressed. In fact, G $\alpha_{12/13}$ -, G $\alpha_{q/11}$ -, or G $\alpha_{i/o}$ -coupled signals induce YAP/TAZ activity, whereas G α_s -coupled signals repress YAP/TAZ activity (Mo et al., 2012; Yu et al., 2012). Rho GTPase are also regulated by mevalonate pathway. Recently in Sorrentino lab it has been shown that statins, by inhibiting the mevalonate biosynthesis, prevent Rho GTPase activation and thus Yki and YAP/TAZ nuclear function (Sorrentino et al., 2014).

Mechanical cues: in vivo, cells are subjected to mechanical stimulation coming from neighbouring cells, the ECM and surrounding biological fluids. These signals influence cell proliferation and migration, and cytoskeletal changes are at the basis of cellular responses to these mechanical stimuli. It has been recently shown that YAP and TAZ are regulated by changes in the actin cytoskeleton in response to mechanical cues experienced by the cell. In particular, cell adhesion, cell geometry, cell shape, cell suspension and extracellular matrix stiffness have been shown to regulate YAP/TAZ nuclear activity in different experimental reports. When cells are grown at low cell density, or on a stiff extracellular substrate, or also on a large adhesive island, conditions where the cell-ECM contact area is broad and the cytoskeleton is subjected to a stronger mechanical stimulation YAP and TAZ are predominantly localized in the nucleus. Conversely, YAP/TAZ effectors translocate to the cytoplasm in response to high cellular density/cell contact, on a soft extracellular substrate or on micropatterned small islands, conditions in which the cell experience a small cell-

ECM contact area and a low mechanical stress. (Dupont et al., 2011; Wada et al., 2011; Zhao et al., 2012; Zhao et al., 2007). YAP and TAZ are not only mechanosensors, but also mechanoeffectors because, once activated, they are able to regulate in turn genes involved in extracellular matrix remodelling (Calvo et al., 2013).

It is still not completely clear how mechanical and biochemical cues experienced by the cell are linked with YAP and TAZ activity. It has been shown that both RHO GTPases and the actin cytoskeleton are able to transduce these upstream signals to YAP and TAZ. In particular, F-actin stabilization and RHO-GTPase activation (depending on the activated G α protein) are able to activate YAP/TAZ, while F-actin destabilization determines YAP/TAZ inhibition. However, the gap between YAP and TAZ and these upstream transducers remains to be fulfilled.

YAP-TAZ effectors and their transcriptional targets

YAP mRNA is ubiquitously expressed in a wide range of mammalian tissues, with the exception of peripheral blood leukocytes (Komuro et al., 2003), it is expressed in all developmental stages from blastocyst to perinatal and it is necessary for a correct and vital embryonic development. TAZ instead shows a later onset, it is present in all the embryonic stages with the exception of blastocyst stage (Morin-Kensicki et al., 2006). YAP and TAZ per se are not able to bind DNA, but they regulate gene targets expression (either by activation or repression) through interaction with transcription factors in a tissue and development specific manner.

By now, several YAP and TAZ interacting proteins have been characterized among which some are able to sequester or post-transcriptionally modify YAP and TAZ (table2) (Chan et al., 2011; Chen and Sudol, 1995; Espanel and Sudol, 2001; Howell et al., 2004; Hsu and Lawlor, 2011; Koontz et al., 2013; Mohler et al., 1999; Rosenbluh et al., 2012; Sudol, 1994; Tsutsumi et al., 2013), others are transcriptional regulators (table3) (Cui et al., 2003; Di Palma et al., 2009; Ferrigno et al., 2002; Hong et al., 2005; Hsu and Lawlor, 2011; Jeong et al., 2010; Kang et al., 2009; Murakami et al., 2005; Wang J et al., 2013; Xiao et al., 2013; Yagi et al., 1999). All the components of the Hippo pathway, from the membrane associated proteins to the cytoplasmic kinase cascade to the final effectors YAP and TAZ, are characterized by specific protein-protein interaction domains, among which the most common are WW domain and the similar SH3 domain, able to bind short peptides that are proline-rich and often terminate with Tyrosine (Y), named PpxY motifs (Sudol and Hunter, 2000).

YAP/TAZ interactors in mammals (regulative proteins)	YAP	TAZ	Human	Mouse, rat
YES (Rosenbluh et al., 2012; Sudol, 1994)	✓		✓	
14-3-3 (Kanai et al., 2000; Vassilev et al., 2001)	✓	✓	✓	
WBP1 and WBP2 (Chen and Sudol, 1995)	✓		✓	
EBP50 (Mohler et al., 1999)	✓		✓	
PP2A (Schlegelmilch et al., 2011)	✓		✓	
AKT (Basu et al., 2003)	✓		✓	
ERBB4 (Komuro, et al., 2003)	✓		✓	
HNRNPU (Howell et al., 2004)	✓		✓	
LATS-1 (Hao, et al., 2008; Lei, et al., 2008)	✓	✓	✓	
C-ABL (Levy, et al., 2008)	✓		✓	
Shp2 (Tsutsumi et al., 2013)	✓	✓	✓	✓
Bmi1 (Hsu, et al., 2011)	✓		✓	
Tgi (Koontz et al., 2013)	✓			✓
Angiomotin (Chan et al., 2011)	✓	✓	✓	
p53BP-2 (Españelet et al., 2001)	✓		✓	
a-catenin (Schlegelmilch et al., 2011)	✓		✓	
SIRT1 (Mao et al., 2014)	✓		✓	
ZO-1 (Remue et al., 2010)		✓	✓	
ZO-2 (Oka et al., 2010; Remue et al., 2010)	✓	✓	✓	✓
PML (Lapi et al., 2008)	✓		✓	

Table 2: YAP/TAZ interactors in mammals (regulative proteins)

There are two major YAP splicing variants with one (YAP1) or two (YAP2) WW domains, but recently, eight different spliced mRNA isoforms of YAP1 gene have been characterized and identified in a panel of human tissues (Gaffney et al., 2012). The different splicing variants of YAP, the different post-transcriptional modifications of YAP and TAZ, and the different chromatin state of target genes may select different repertoires of proteins in transcriptional complexes and affect the gene expression program in a developmental and tissue-specific manner (Beyer et al., 2013; Reginensi et al., 2013; Slattery et al., 2013).

The transcription factors with which YAP and TAZ cooperate are directly involved in control of cell

proliferation/survival or apoptosis, like TEAD (Chan et al., 2009; Mahoney et al., 2005; Ota and Sasaki, 2008; Vassilev et al., 2001; Zhang et al., 2009; Zhao et al., 2008) and p73 transcription factors, (Strano et al., 2001) or are components of other signalling pathways as Wnt, EGFR, JAK/Stat, BMP-TGFbeta involved in embryonic development and adult tissue homeostasis. For example, it has been shown that YAP/TAZ interact with Smad proteins (Smad1, Smad2, Smad3) to enhance the transcription of genes responsive to BMP-TGFbeta signalling (Alarcon et al., 2009; Schlegelmilch et al., 2011; Varelas et al., 2010). Other transcriptional targets are represented by components of the Hippo pathway.

YAP/TAZ interactors in mammals (transcription factors)	YAP	TAZ	Human	Mouse, rat
RUNX2 (Cui et al., 2003; Yagi et al., 1999)	✓	✓	✓	✓
TTF-1/Nkx-2.1 (Di Palma et al., 2009)		✓	✓	✓
TP73 (Strano et al., 2001)	✓		✓	
TBX5 (Murakami et al., 2005)	✓	✓	✓	✓
PPARγ (Hong et al., 2005)		✓		✓
MyoD (Jeong et al., 2010)		✓	✓	✓
Glis3 (Kang et al., 2009)		✓		✓
SMAD1, SMAD2, SMAD3 (Alarcon et al., 2009)	✓	✓	✓	
SMAD7 (Ferrigno, et al., 2002)	✓		✓	
PAX8 (Di Palma et al., 2009)		✓	✓	✓
Bmi1 (Hsu and Lawlor, 2011)	✓		✓	
CREB (Wang et al., 2013)	✓			✓
TEAD1-4 (Vassilev et al., 2001) (Zhao et al., 2008; Mahoney et al., 2005; Chan et al., 2009; Zhang et al., 2009)	✓	✓	✓	✓
c-myc (Xiao et al., 2013)	✓		✓	✓

Table 3: YAP/TAZ interactors in mammals (transcription factors).

Tumor tissue-cell line	Yap upregulation	TAZ upregulation	Lats1/Lats2 downregulation	Mst1/Mst2/Sav1 downregulation	human	mouse
ovary (Steinhardt et al., 2008; Hall et al., 2010; Zhang et al., 2011)	v				v	
prostate (Zhao et al., 2012)			v		v	
intestine (Wang et al., 2013; Steinhardt et al., 2008; Zhou et al., 2011a; Yuen et al., 2013; Wierzbicki et al., 2013)	v	v	v	v	v	v
lung (Steinhardt et al., 2008) (Su et al., 2012) (Wang et al., 2010) (Zhou et al., 2011b) (Lau et al., 2014)	v	v			v	v
breast (Cordenonsi et al., 2011; Overholtzer et al., 2006; Lamar et al., 2012; Wang et al., 2012; Chan et al., 2008; Bartucci et al., 2014; Takahashi et al., 2005)	v	v	v		v	v
liver (Camargo et al., 2007; Dong et al., 2007; Lee et al., 2010; Lu et al., 2010; Zender et al., 2006; Song et al., 2010; Zhou et al., 2009; Xu et al., 2011)	v			v	v	v
Esophageal cancer (Muramatsu et al., 2011)	v				v	
Cutaneous melanoma (Nallet-Staub et al., 2013)	v	v			v	v
skin basal cell carcinoma (Quan et al., 2014)	v				v	
kidney (Matsuura et al., 2011)				v	v	
bladder (Liu et al., 2013)	v				v	

Table 4: Tumor tissues or tumor cell lines where YAP/TAZ are overexpressed or hyperactivated.

The dual role of YAP as an oncoprotein or an oncosuppressor

A lot of studies supported the functional conservation of the core Hippo pathway components between *Drosophila* and mammals in the control of cell proliferation.

When Hippo kinase pathway is inactive, YAP and TAZ enter the nucleus and affect transcription of different sets of target genes in a tissue and developmental specific manner (Beyer et al., 2013; Slattery et al., 2013).

Increasing evidences showed that the transcriptional outcome in response to YAP/TAZ activation can be opposite.

In mammals, it has been shown that YAP transcriptional coactivator can function either as an oncogene, or as a tumor suppressor, depending on the signals to which cells are subjected and on the transcription factors with which YAP is associated. The emerging and intriguing dual role of YAP and the mechanisms determining the two exclusive cellular responses (pro-proliferative or pro-apoptotic) are still not entirely understood as they were built on classic studies performed in different cell types and tissues.

Here, we will discuss experimental evidences showing YAP as an oncogene or as an oncosuppressor.

YAP as an oncogene

There are several evidences supporting a pro-proliferative and pro-oncogenic role of YAP (and TAZ) in mammalian systems. In humans, YAP is present in the 11q22 amplicon that is amplified in a lot of solid tumors (Baldwin et al., 2005; Bashyam et al., 2005; Dai et al., 2003; Hermesen et al., 2005; Imoto et al., 2002; Imoto et al., 2001; Lambros et al., 2005; Overholtzer et al., 2006; Snijders et al., 2005; Weber et al., 1996) (Table 4).

The syntenic chromosomal region in mouse contains YAP gene that is amplified in mammary and liver tumors (Overholtzer et al., 2006; Zender et al., 2006).

Ectopic expression or hyperactivation of YAP promotes cell growth and induces oncogenic transformation and epithelial-mesenchymal transition (EMT) that is often associated with metastasis (Lamar et al., 2012; Lau et al., 2014; Nallet-Staub et al., 2013; Overholtzer et al., 2006; Zhao et al., 2009; Zhao et al., 2008).

In mouse, transgenic YAP overexpression or liver-specific knockout of Mst1/2 and Sav1 increases the number of stem/progenitor cells and determines liver overgrowth in a reversible manner, ultimately leading to hepatocellular carcinoma (HCC) (Camargo et al., 2007; Dong et al., 2007; Lee et al., 2010; Lu et al., 2010; Song et al., 2010; Zhou et al., 2009).

Consistently, a lot of human cancers show overexpression or hyperactivation of nuclear YAP or TAZ or downregulation of Lats1/2, Mst1/2 or Sav1 function (Dong et al., 2007; Hall et al., 2010; Jiang et al., 2006; Matsuura et al., 2011; Muramatsu et al., 2011; Nallet-Staub et al., 2013; Quan et al., 2014; Seidel et al., 2007; Steinhardt et al., 2008; Su et al., 2012; Takahashi et al., 2005; Wang L et al., 2013; Wang et al., 2012; Wang et al., 2010; Wierzbicki et al., 2013; Xu et al., 2011; Xu et al., 2009; Yuen et al., 2013; Zender et al., 2006; Zhao et al., 2007; Zhou Z et al., 2011) see also table 4. Moreover, overexpression or hyperactivation of YAP and TAZ have been associated with poor prognosis and shorter survival times for patients in several human cancers (Hall et al., 2010; Liu JH et al., 2013; Muramatsu et al., 2011; Wang et al., 2010; Xu et al., 2009; Zhang et al., 2011). It has been also shown that Mst1/2 and Sav1 knockout, or YAP activation expanded the stem and the progenitor cell population in the intestine and in the skin in mouse (Lee et al., 2008; Schlegelmilch et al., 2011; Zhou D et al., 2011). YAP has been shown to contribute also to the expansion of neuroprogenitor cells (Cao et al., 2008). In addition, YAP has been found to be upregulated in mouse Embryonic Stem cells (mES) and in induced pluripotent stem cells (iPS) and to contribute to their stemness by binding and activating a large number of genes known to be important for stem cell maintenance (Lian et al., 2010).

TAZ is overexpressed in breast cancer stem cells and is required to maintain their self-renewal capacity, tumorigenicity and ability to promote the formation of metastasis (Bartucci et al., 2014; Chan et al., 2008; Cordenonsi et al., 2011). Moreover, YAP and TAZ have been recently found to be upregulated in mouse wounds and to be required for wound closure (Lee et al., 2014). Based on these results, YAP and TAZ are defined as oncogenes and as "stemness genes" (Ramalho-Santos et al., 2002).

TEAD transcription factors guides YAP and TAZ onto pro-proliferative genes (Chan et al., 2009; Lamar et al., 2012; Mahoney et al., 2005; Zhang et al., 2009; Zhao et al., 2008).

YAP as a tumor suppressor

We originally showed that the tumor suppressor p73 protein, which belongs to the p53 family, has been shown to guide YAP onto pro-apoptotic targets. These findings together with other evidences from diverse labs indicated that YAP might behave as a tumor suppressor, in particular upon DNA damage signalling and serum deprivation (Lapi et al., 2008; Oka et al., 2008; Strano et al., 2005; Strano et al., 2001; Yuan et al., 2008). p73-YAP interaction is increased upon DNA damage (Strano et al., 2005), where it has been

shown to be phosphorylated by c-Abl that stabilizes both YAP and p73 and increases YAP/p73 interaction (Levy et al., 2008). On the other hand, p73-YAP interaction is inhibited upon Akt-mediated YAP phosphorylation (Basu et al., 2003). p73 is post-transcriptionally stabilized by YAP binding that competes with the E3 ubiquitin ligase ITCH, thereby preventing proteasomal degradation of p73 (Levy et al., 2007). YAP binding also induces p73 acetylation and transcriptional activity by recruiting the p300 acetyltransferase to target genes (Strano et al., 2005). Another oncosuppressor, PML (Promyelocytic leukemia protein) has been shown to act together with YAP and p73 as a mediator onto several proapoptotic target genes following DNA damage by physically interacting with both p73 and YAP (Bernassola et al., 2004; Lapi et al., 2008). PML is a key component and organizer of nuclear compartments termed nuclear bodies (NBs) implicated in processes such as transcriptional regulation, genome stability, response to viral infection, metabolism, apoptosis, and cell cycle control (reviewed in Gamell et al., 2014). It has also been proposed that PML partially collaborates with YAP and p73 in the proapoptotic response induced by DNA damage by several self-reinforcing mechanisms. First, YAP requires PML and NBs localization to coactivate p73 and, conversely, YAP and p73 are required for PML accumulation and PML-NB formation in response to DNA damage. Second, PML stabilizes YAP from proteasomal degradation by inducing its sumoylation and its recruitment into PML-nuclear bodies, where it collaborates with YAP and p73 onto target genes. Third, PML itself is a transcriptional target of YAP-p73-PML complex (Lapi et al., 2008; Strano et al., 2005).

Interestingly, it has been shown an important role for YAP in the regulation of cellular senescence in a functional cooperation with PML and p53 (Fausti et al., 2013; Xie et al., 2013).

Finally, while in many solid cancers YAP behaves as an oncogene and is upregulated or hyperactivated (see above), in hematologic malignancies, including lymphomas, leukemias and multiple myelomas YAP is deleted or downregulated. Lower YAP expression level correlates with poorer prognosis and shorter survival of patients (Cottini et al., 2014). In the context of hematologic malignancies, YAP downregulation is a mechanism by which cells escape apoptosis in the presence of DNA damage. In fact, in normal hematologic cells YAP is phosphorylated by c-abl that stabilizes YAP/p73 interaction and increases their transcriptional activity onto pro-apoptotic genes in the presence of DNA damage (Levy et al., 2007; Levy et al., 2008), while in malignant cells, where YAP is downregulated or absent, the c-Abl/p73/YAP axis

is disrupted (Cottini et al., 2014). Collectively, these observations do not classify YAP as a real tumor suppressor, but as a transcriptional co-activator that can directly or indirectly regulate different tumor suppressor pathways (as p53 family or PML).

A better understanding of the role of the Hippo pathway in tumorigenesis assessed in different experimental and physiological/pathological conditions would be important for a more specific characterization of prognostic factors in cancer and for the development of anti-cancer therapies that often need to be adapted to the type of disease and to the individual patient.

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